Right fronto-parietal white matter disruption contributes to speech impairments in amyotrophic lateral sclerosis

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Abstract

Introduction: Non-linguistic properties of speech are widely heterogeneous and require complex neurological integration. The association between white matter integrity and the severity of dysarthria was investigated in a group of patients diagnosed with amyotrophic lateral sclerosis (ALS).

Methods: Thirty-six patients diagnosed with amyotrophic lateral sclerosis completed a magnetic resonance imaging protocol inclusive of diffusion-weighted images. A clinical assessment of pneumo-phono-articulatory abilities was conducted for each patient, and a composite score of residual speech capacity was calculated. Tract-Based Spatial Statistics was carried out to model the potential association between residual speech capacity and microstructural properties of white matter (fractional anisotropy, mean and radial diffusivity).

Results: A significant negative association was found between residual speech capacity and mean diffusivity in a large white matter cluster located in frontal, parietal and right temporal regions. These subcortical areas were characterised by pathological microstructural disruption, as revealed by *post hoc* analyses.

Conclusions: Non-linguistic aspects of speech are associated with microstructural integrity of frontal, parietal and right temporal white matter in amyotrophic lateral sclerosis. Such mapping is consistent with the centres responsible of volitional control of speech and sensory feedback during non-linguistic speech production.

Keywords

motor neurone disease; dysarthria; mean diffusivity; radial diffusivity; fractional anisotropy; DTI

Introduction

The term ‘dysarthria’ identifies a wide and heterogeneous set of disorders of diverse complexity, which interfere with speech production [1]. Several neurological conditions present with dysarthric speech at some stage of their clinical progression. This is the case of Amyotrophic Lateral Sclerosis (ALS). In ALS, the main putative cause of dysarthria is the impoverishment of neurons that support the motoric aspects of speech. Although the occurrence of dysarthria is typically due to depletion of the lower motor neurons (i.e., brainstem nuclei), ALS (in its typical course) is characterised by a concomitant depletion of upper motor neurons (i.e., motor cortex). As a consequence, as the disease progresses, speech deficits become characterised by both spastic and flaccid features, which are the result of the involvement of both, upper and lower motor neurons [2]. These symptoms are clinically distinguishable from aphasia and apraxia of speech, as the former affects linguistic abilities (i.e., phonology, semantics, syntax) in comprehension or production, while the latter consists of impaired articulatory planning of speech production [3]. Although ALS may affect motor and non-motor systems, the presence of speech apraxia is relatively rare [4,5]. Albeit not uncommon [6,7], the presence of central linguistic deficits, i.e., receptive/productive aphasia, in ALS is certainly less remarkable than central dysarthria. In fact, world experts consider the presence of dysarthria as an essential feature in the clinical profile of ALS patients [8]. A careful analysis of dysarthric speech may yield important clinical information on the degree of neural disruption caused by the disease. Nevertheless, the neurobiological substrate of dysarthria in ALS has yet to be fully clarified.

Speech production is a multidimensional ability dependent on neurological control and integration of various aspects: respiration, phonation, vocal resonation, and motor articulation. Such an integration is achieved at a neural level, and is thought to be sustained by a network of regions that include the insula, Broca’s area, basal ganglia, the cerebellum,

and the primary and supplementary motor cortices, each of them taking on a specific computational role [9]. In normal conditions, the interplay of these regions is granted by their respective afferent and efferent white matter (WM) fibres. It derives that disruption of such connective tracts may impair the neurological integration of the computational units engaged in speech production. We had previously tested this hypothesis using voxel-based morphometry in a sample of ALS patients without respiratory dysfunction, and a positive association between retained speech production abilities and WM volume of motor/premotor and cerebellar areas was found [10]. The investigation of the sole volumetric features of WM, however, underestimates the presence of subtle microscopic abnormalities that may precede the occurrence of atrophy.

In this study we modelled the association between the microstructural properties of WM in a sample of patients with ALS, and their retained pneumo-phono-articulatory competence, as measured by formal tests of dysarthria that are widely used by speech therapists in clinical settings.

Materials and Methods

Participants

Fifty consecutive in-patients from the IRCCS Fondazione Ospedale San Camillo (Venice, Italy) with a working diagnosis of ALS made between February 2012 and November 2015 were considered for inclusion. Diagnoses were reached following the application of the El Escorial criteria [11,12], and clinical confirmation was sought by means of regular clinical follow-up assessments, during which one of the participants was excluded because of diagnostic uncertainties (i.e., possible spinal muscular atrophy with a tardive onset). The

remaining patients were invited to take part in this research. Cognitive profiling was carried out for each candidate and the presence/absence of cognitive impairment (decided based on a consensus between neurologist and neuropsychologist) was verified with a valid informant who had monitored the patient before and during hospitalisation. Speech assessment was not completed for six patients because of medical incompatibilities (e.g., tracheostomy) or presence of objective cognitive impairment. Additionally, seven patients were unable to complete the MRI scan. As a result, thirty-six cognitively-normal participants (28 right-handed) were included in the final sample (**Fig. 1**). Their main demographic and clinical characteristics (including the revised version of the Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) [13], and the Functional Independence Measure [14] scale) are summarised in **Table 1**.

A group of 36 healthy controls was also included in this study. These were of comparable age to the group of patients (*mean* 66.56 years, *sd* 10.77), and served to characterise the group of patients more in detail.

--- Please include **Table 1** and **Fig. 1** about here ---

Speech Assessment

The assessment of pneumo-phono-articulatory properties of speech was carried out by an experienced speech therapist. In order to minimise the influence of external factors (e.g., excessive salivation or swallowing difficulties) on performance, the assessment was carried out at a moment in which speech was unaffected by intervenient variables. Measurements were acquired following the procedures described in the Robertson's Profile Test of dysarthria [15] that focuses on the perceptual properties of speech production. Trials number

1, 2, and 4 were selected from the original testing battery, and were administered to each patient based on the transposition into Italian published by Fussi and Cantagallo [16]. These three sub-tests served to measure proxies of pneumonic, phonatory, and articulatory capacity, respectively:

- Trial 1 - Maximal Expiratory Duration Rate: the capacity to sustain an '/s/' sound in a prolonged way (measured in seconds).
- Trial 2 - Maximal Phonatory Duration Rate: the capacity to sustain an '/a/' sound (measured in seconds).
- Trial 4 - Diadochokinetic Rate (rapid sound articulation). Along six trials, patients were asked to repeat in rapid succession the following sequences, as quickly and as steadily as possible: '/u-i/', '/pa/', '/ta/', '/ka/', '/kala/', '/ptk/'. The number of correct sound sequences produced over the first five seconds was extracted for each of the six sequences, and a mean of the six scores was calculated.

The three indices were *z*-transformed, which did not breach the assumption of normality (Kolmogorov-Smirnov test: all p values > 0.05). Significant positive Pearson's correlations were found among the three scores (all r coefficients > 0.5). To obtain a single measure of retained pneumo-phono-articulatory capacity, a composite index was computed, averaging the three *z-scores* for each patient.

MRI Acquisition, Processing and Modelling

The MRI protocol was acquired on a 1.5 T system (Achieva; Philips Healthcare, Best, The Netherlands), including the following acquisitions:

1) 3D Turbo-Field Echo T1-weighted: repetition time (TR) 7.5 s, echo time (TE) = 3.52 ms, matrix = 228 × 227, in-plane FOV = 250 × 168 mm^2, slice thickness 1.2 mm, gap = 1 mm;

2) Turbo Spin Echo T2-weighted: TR = 5000 ms, TE = 100 ms, matrix = 400 × 296, FOV = 240 × 129 mm^2, slice thickness 4.2 mm, gap = 1 mm;

3) Fluid-attenuated turbo inversion recovery (FLAIR SPIR SENSE): TR = 8000 ms, TE = 125 ms, inversion time (TI) = 2300, matrix = 260 × 232, FOV = 148 × 240 mm^2 and slice thickness = 4.5 mm, gap = 0.45 mm;

4) Single-shot echoplanar diffusion-weighted (DW) SENSE: TR = 8.28 s, TE = 70 ms, b factor = 600 s/mm^2, resolution = 1.67 × 1.67 × 3 mm^3, matrix = 96 × 96, slice thickness = 3 mm, no gap. This last sequence collects one image with no diffusion weighting (b_0) and 32 images with diffusion gradients applied in 32 noncollinear directions.

Anatomical images were reviewed by a neuroradiologist to rule out the presence of brain abnormalities suggestive of major aetiological entries that would otherwise account for the presence of neurological symptoms other than those expected from a diagnosis of ALS (e.g., ALS mimic syndromes).

Diffusion data were processed using tools from the FMRIB Software Library (FSL, www.fmrib.ox.ac.uk/fsl/fslwiki/). After eddy-current correction the diffusion tensor was estimated in a voxel-wise fashion [17].

The 36 maps of fractional anisotropy (FA) were processed following the Tract-Based Spatial Statistics (TBSS) procedural steps [18]. Firstly, FA images were eroded to exclude outlier voxels from diffusion tensor fitting. The most representative subject was identified and used as a template for non-linear normalisation. Subsequently, the images were aligned to the MNI152 standard space and a mean FA skeleton image was created. A threshold of 0.2 was applied to the mean FA skeleton image to exclude grey matter and cerebrospinal fluid voxels

and create a binary skeleton mask. Finally, individual FA, mean diffusivity (MD) and radial diffusivity (RD) images were projected onto the FA skeleton and voxel-wise statistics were carried out within the skeleton. In particular, voxel-by-voxel statistical models were set up to infer the significant positive and negative association between FA/MD/RD and the composite score of retained speech competence. For this purpose, contrast matrices were created including the speech composite score as variable of interest and the ALSFRS-R score as nuisance variable, to control for global levels of disability. In a second set of models, a respiratory sub-score constructed based on the last three questions of the ALSFRS-R scale was added as further covariate to control for a general index of respiratory failure. Using the FSL tool 'randomise', permutation-based statistical analyses were carried out [19] on the FA, MD and RD skeletonised images for statistical inference, with 5000 permutations for each contrast. The resultant statistical maps were thresholded at $p < 0.05$, corrected for multiple comparisons using the Threshold-Free Cluster Enhancement method [20]. Coordinates were interpreted following the JHU WM tractography atlas [21].

Results

Of the clinical variables included in **Table 1**, the speech composite was significantly correlated with the total score and with the bulbar sub-score of the ALSFRS-R (both Pearson's *r* and Spearman's $\rho = 0.4$, all *p-values* < 0.05). No association was found with age, disease duration, or level of formal education.

No significant association was found between retained speech competence and FA. Conversely, a large and widespread pattern of negative association emerged with MD and RD values mainly in fronto-parietal areas (**Table 2**; **Fig. 2**). The composite score of speech

competence was associated with MD in a large territory covering a number of fibre tracts, bilaterally: superior longitudinal, inferior fronto-occipital and uncinate fasciculi, corpus callosum, cingulum and corticospinal tract. Additionally, a significant association was also found in the right inferior longitudinal fasciculus (**Fig. 3**). Results from the analysis of RD maps revealed an association mainly in the left superior longitudinal fasciculus and in the right inferior longitudinal fasciculus. The inclusion of the ALSFRS-R respiratory sub-score led to a comparable pattern of findings. To understand the extent to which each of the three speech parameters contributed to the results found in the map of MD, separate post hoc models were run to compute the association between MD and, separately, expiratory, phonatory and diadochokinetic rate. The outcome of these post hoc analyses indicated that the maximum expiratory rate and the diadochokinetic rate were associated with MD following a pattern very similar to that of the global composite score. Vice versa, the maximum phonatory rate did not yield any significant result when analysed as a stand-alone variable (**Supplementary Material**).

To clarify whether these significant associations were found in regions of normal or abnormal WM integrity, a *t*-test was run to compare the maps of DTI indices between the group of patients and the group of 36 healthy controls. The results showed that patients had widespread bilateral areas of decreased FA and increased MD/RD especially in the corticospinal tract, the body of the corpus callosum and the superior longitudinal fasciculus, but also in all WM tracts where an association with retained speech abilities had been found in the group of patients.

--- Please include **Table 2**, **Fig. 2** and **Fig. 3** about here ---

Discussion

Dysarthria is a major symptom of ALS [8], but the neural mechanisms underpinning retained pneumo-phono-articulatory competence in ALS patients is still poorly understood. Since the primary degenerative damage in ALS brains is the death of upper and lower motor neurons, their axonal disruption resulting in reduced WM integrity is likely to play a major role in determining dysarthria, a clinical feature dominated by impaired muscle control. Consistently with the 'disease-spreading hypothesis', however, ALS is also characterised by non-motor symptoms [22]. As a consequence, non-motor neurological components may also play a crucial role in this symptom. One major system of interest is the neurological integration of motor and non-motor functions required for speech execution [9]. Although of difficult operationalisation, speech-driven neural integration is essential for correct speech production. In the current study, we used a composite score derived from indices of articulation speed, and phonatory/expiratory endurance to explore, in an unbiased way, its potential pathophysiological substrate in terms of WM abnormalities and, ultimately, brain disconnection. We had previously demonstrated that WM volumes in the motor and premotor subcortical WM, bilaterally, and in the right cerebellum correlate with the severity of speech production deficits in ALS patients [10]. In this study, based on more sensitive measures of WM integrity, we identified the fronto-parietal WM as the core region the microscopic abnormalities of which are associated with speech impairments in patients with ALS (the higher MD/RD, the worse the properties of speech). To interpret these findings appropriately, it is important to clarify which neural systems sustain such skills. It is well established that language computations tend to be highly lateralised in the left cerebral hemisphere. Speech production, however, is at least partially independent from language abilities, as it relies on the generation of sounds that may be devoid of any distinctive lexical-

semantic, morpho-syntactic, or phonemic content. Such a volitional control of voice is sustained by a complex set of interlaced systems in which both cerebral hemispheres are involved [23]. A detailed focus on the neuroanatomy of each subcomponent of the composite score we used here, allows us to exploit various interpretational possibilities.

The neural centres responsible for automatic respiratory rhythms (i.e., brain stem nuclei) are part of larger bilateral networks with two specific components (i.e., the Bötzinger complex and the caudal ventral respiratory group) that control the phase which follows inspiration and post-inspiration, and activate the expiratory motor neurons [24]. Vocal-related expiration, however, is the result of volitional respiration, which is associated with bilateral activation of the primary sensorimotor, supplementary motor and premotor cortices, the cerebellum, the thalamus, the caudate nucleus and pallidum [25]. A similar hierarchy sustains phonation, which is regulated by the interplay of a 'lower-level' centre that controls vocal patterns (located in the reticular formation) [26], and a 'higher-level' centre that involves the cerebral cortex. When using a paradigm of pitch modulation (arguably involving limited articulation) to investigate these functions, phonatory control is associated with the activation of a set of cortical and subcortical areas, with some specific regions involved in either pitch increase or decrease [27]. When controlled for expiration, however, phonation is centred in the superior temporal gyrus and insula, bilaterally. This was found regardless of whether phonation was delivered at a comfortable pitch or, purportedly, at a high pitch [28]. A major computational component behind both expiration and phonation is certainly laryngeal control, which is achieved via bilateral activation of a small region located within the primary motor cortex, dorsolaterally. In an fMRI study it was found that this region controls both glottal stops responsible for air flow (expiration) as well as phonation [29]. This area is connected with a wide range of regions including numerous subcortical structures and various premotor, prefrontal and, to a lesser extent, parieto-temporal regions [30,31].

Finally, of the three speech sub-components investigated here, articulation is that associated with the widest variability in terms of delivery. In contrast, phonation and respiration, in a sense, control a more 'uni-directional' set of effectors. Motor control in support of articulation is more extensive than simple laryngeal control, and results in activations of areas associated with motor preparation and execution that are centred in the sensorimotor cortex, supplementary motor area, cerebellum, midbrain, thalamus, lentiform nucleus and insula, with a preponderant involvement of the supplementary motor cortex during preparation, and cerebellum during execution [32-34].

The above experimental evidence reported in the literature highlights the role of prefrontal, premotor, motor and parietal cortex in support of volitional expiratory, phonatory and articulatory control. Consistently, we found that in our cohort of ALS patients a large and widespread association exists between the index of retained speech and MD values, predominantly in the WM proximal to premotor, prefrontal and parietal areas. This pattern, however, was not replicated when phonation was analysed as a stand-alone variable. A comparable but more spatially constrained pattern was found in association with RD. RD is the metric of WM integrity that is most altered in ALS [37]. The fronto-parietal pattern in this case was lateralised to the left hemisphere, suggesting that there might be mechanistic differences between the two hemispheres in the way damage to WM integrity associates to dysarthria.

Aside from a possible regional specialisation shown by prefrontal and parietal tracts, the association might have in part emerged as the result of a certain vulnerability observed in these regions. Prefrontal and parietal deposition of TDP-43 pathology, in fact, is detectable in ALS patients regardless of cognitive impairment [38], indicating that pathological changes in these regions are not uncommon in ALS regardless of speech difficulties.

Although the findings presented with a bilateral topography, an asymmetrical pattern emerged in the temporal lobe, with the sole right inferior longitudinal fasciculus showing a significant association with MD and RD. The right temporal lobe is preferentially involved (as opposed to the left temporal lobe) in '*non-linguistic processing of sounds'* [35]. On this note, the sub-tests extracted from the Robertson's Profile Test of dysarthria and used in this study are based on tasks devoid of any linguistic features, and therefore unlikely to be influenced by language lateralisation. For this reason, handedness was not included in any of the statistical models. Moreover, the requests of these tasks are not automated, but require some degree of control. In these conditions, speech production is necessarily accompanied by concurrent processing of sensory feedback [36]. This would be even more relevant in patients with ALS, who suffer from dysarthria as a central symptom. We propose that the involvement of the right hemisphere is partially the result of less efficient sensory feedback. This would be supported by right temporal areas and by their innervating fibres, where a significant association between loss of tissue integrity and patients' deficits was found.

Interestingly, no association was found with FA WM values in agreement with previous literature. In fact, it has been previously argued that the WM degeneration occurring in ALS typically results in MD but not FA changes [39].

This study is not free from limitations. Although the multi-componential nature of speech production is conveniently and ecologically operationalised as a function of a single variable, the composite score we implemented reflects multiple processes: respiration, phonation, articulation, and neurological integration. It cannot be determined, therefore, whether there was a main drive behind the statistical association we found. On the other hand, a recent study reported that decline in articulatory (but not respiratory or phonatory) capacities is strictly associated with disease progression [40]. It is possible that different instruments (i.e., experimental, rather than clinical measures) of speech may allow a specific separation of its

various components and further clarify the mechanisms of integration. Moreover, it is also possible that the use of MRI sequences obtained with stronger magnetic fields may have led to slightly different patterns of findings.

In conclusion, speech impoverishment is associated with increased MD and RD in bilateral frontal-parietal and right temporal WM regions. This is thought to reflect the pathological WM changes observed in ALS, and is in part the result of failure in sensory feedback supporting speech.

Compliance with ethical standards

Conflicts of interest

The authors report no disclosures.

Ethical approval

All the procedures conducted with the participants of this study were carried out in compliance with the Declaration of Helsinki.

Informed consent

Ethical approval was granted by the Institutional Ethics Review Board, reference nº ll/09, version 2. All participants provided their written informed consent.

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Figure Captions

Fig. 1

Flowchart illustrating sample selection.

Fig. 2

Negative correlations between the residual pneumo-phono-articulatory competence and indices of microstructural white matter integrity: A. mean diffusivity; B. radial diffusivity ($p < 0.05$).

Fig. 3

Negative correlations between the residual pneumo-phono-articulatory competence and mean diffusivity ($p < 0.05$) with detail of the inferior longitudinal fasciculus in the left (A) and right temporal lobe (B). Slices in the MNI spaces are $x = -36$ (A) and $x = 36$ (B).

Fig. 1

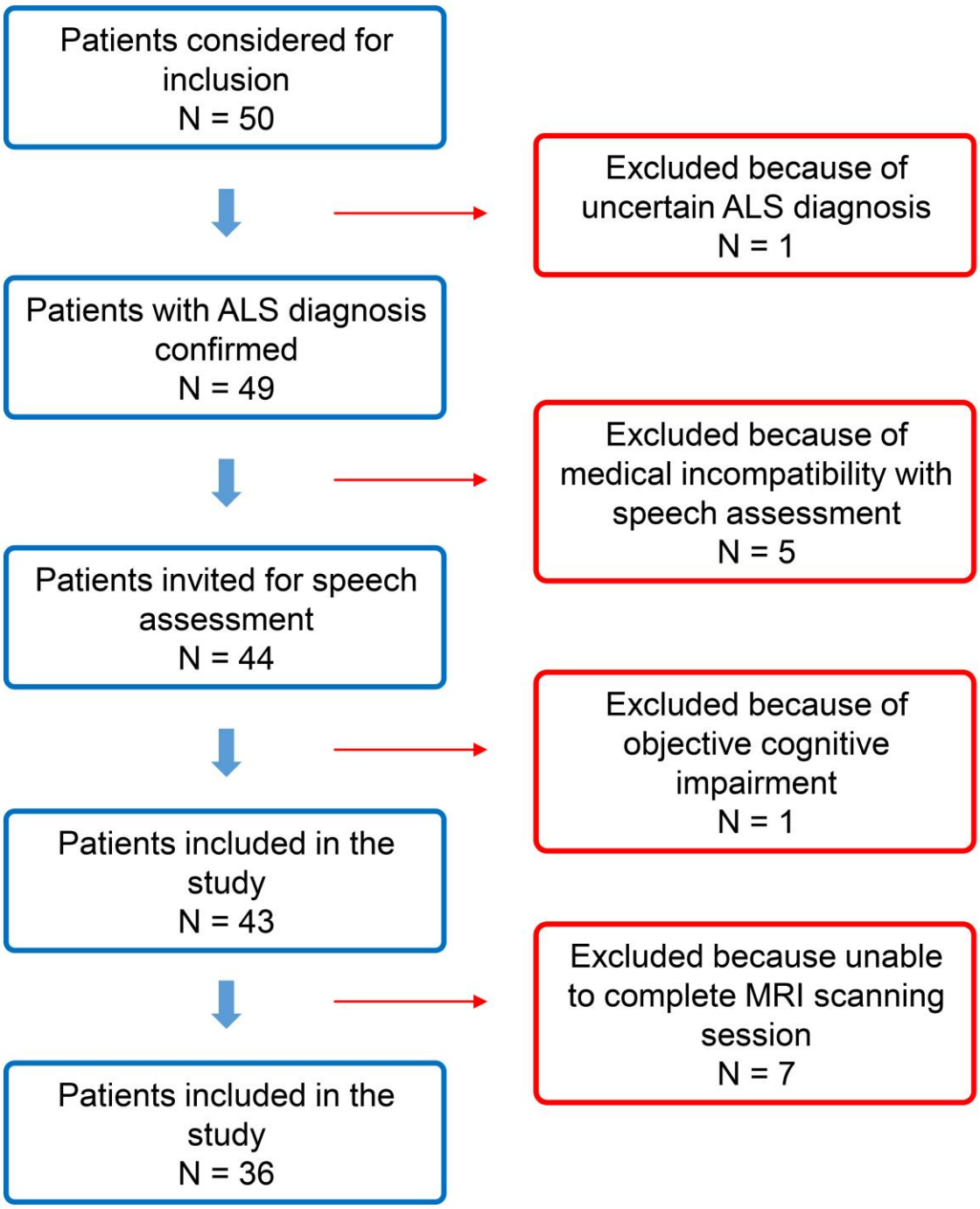
Patients considered for inclusion
N = 50
Excluded because of uncertain ALS diagnosis
N = 1
Patients with ALS diagnosis confirmed
N = 49
Excluded because of medical incompatibility with speech assessment
N = 5
Patients invited for speech assessment
N = 44
Excluded because of objective cognitive impairment
N = 1
Patients included in the study
N = 43
Excluded because unable to complete MRI scanning session
N = 7
Patients included in the study
N = 36

Fig. 2

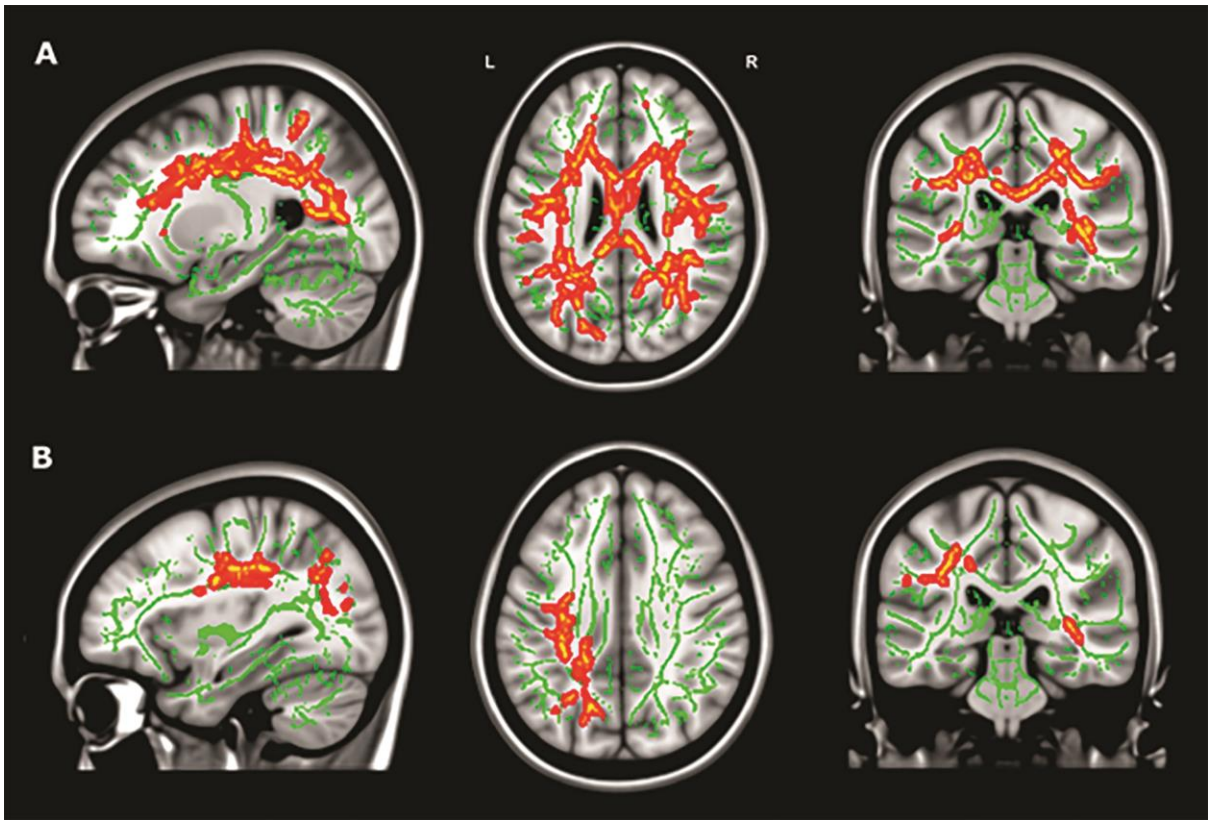

Fig. 3

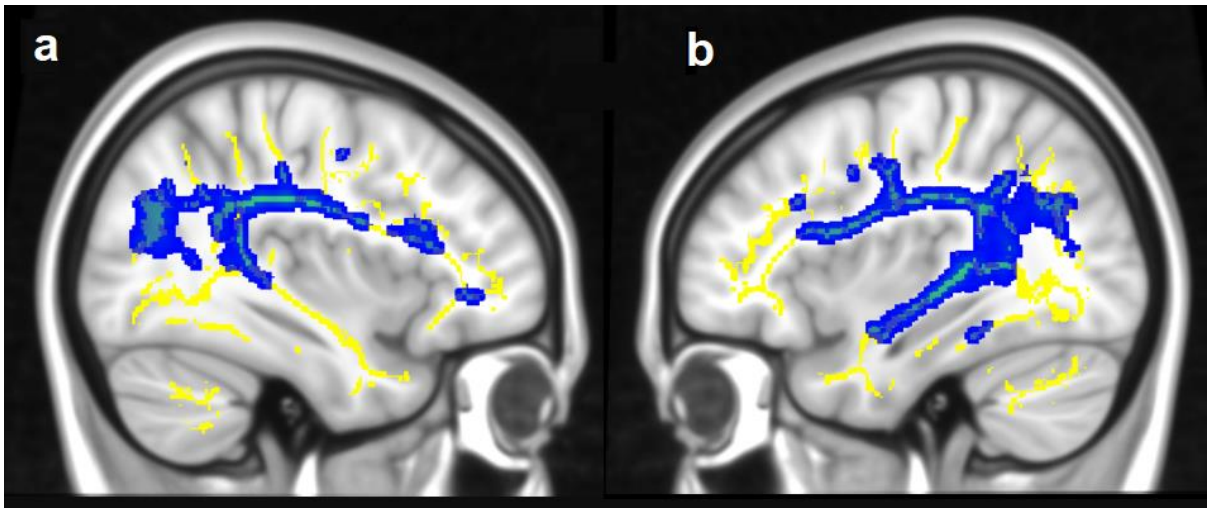

Table 1. Demographic and clinical characteristics of the sample

Variable	Mean	SD	Min	Max
Age (years)	60.36	12.18	26	79
Education (years)	8.89	3.91	5	19
ALSFRS-R - Total Score	32.36	5.48	21	42
ALSFRS-R - Bulbar Sub-score	9.58	1.78	6	12
FIM†	72.53	13.64	35	88
Disease Duration (months)	36.53	14.94	16	68
Expiratory Duration Rate (s)	13.47	8.90	1	38
Phonatory Duration Rate (s)	13.78	7.63	2	33
Diadochokinetic Rate (num. sequences)	10.86	4.58	1.80	20.50

SD: Standard deviation; ALSFRS-R: Amyotrophic lateral sclerosis functional rating scale – revised version; FIM: Functional independence measure. †A FIM ≤91 has been established by the regional legislation as cut-off for admission to hospitalised care

Table 2 Negative correlations between the residual pneumo-phono-articulatory competence and indices of microstructural white matter integrity ($p < .05$). Top statistical peaks per each independent cluster are reported

Cluster extent	r	Side	*t* value	MNI coordinates		
				x	*y*	*z*
			Mean diffusivity			
19393		R	5.22	18	-49	34
		L	5.06	-26	-27	42
		R	4.99	16	-53	32
		R	4.88	36	-1	23
		R	4.79	41	1	19
		L	4.77	-30	16	22
107		L	4.25	-39	39	-6
		L	3.43	-34	40	-2
		L	3.24	-33	43	-3
		L	3.23	-40	42	-6
		L	2.97	-28	39	-3
		L	2.74	-30	39	2
41		R	3.40	18	-70	43
		R	3.37	16	-70	44
		R	2.73	13	-70	44
		R	2.58	13	-73	44
		R	1.98	18	-65	41
			Radial diffusivity			
1777		L	4.61	-53	-29	30
		L	4.57	-28	-60	36
		L	4.52	-37	-8	26
		L	4.26	-20	-71	34
		L	4.22	-33	-7	27
		L	4.21	-35	-24	31
291		L	3.60	-36	-74	27
		L	3.52	-39	-67	18
		L	3.27	-36	-72	28
		L	3.26	-34	-67	17

	L	3.16	-36	-71	26
	L	3.02	-37	-70	22
196	L	3.99	-19	-48	43
	L	3.78	-19	-47	45
	L	3.27	-18	-43	53
	L	2.95	-19	-47	49
	L	2.81	-19	-51	42
	L	2.75	-15	-54	47
136	R	4.20	34	-30	4
	R	3.98	34	-21	-2
	R	3.60	33	-18	-2
	R	3.48	34	-22	-4
	R	3.21	31	-25	-4
	R	2.83	38	-33	-2
54	L	3.92	-24	-51	56
	L	3.43	-24	-53	56
	L	3.32	-25	-54	59
	L	2.95	-25	-51	59
	L	2.61	-22	-50	51
	L	2.45	-23	-51	53

Coordinates are expressed in the Montreal Neurological Institute space